Reference: *Biol. Bull.*, **152**: 382–391. (June, 1977)

DEVELOPMENT OF THE DIMORPHIC CLAW CLOSER MUSCLES OF THE LOBSTER, *HOMARUS AMERICANUS*: II. DISTRIBUTION OF MUSCLE FIBER TYPES IN LARVAL FORMS

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The bilateral closer muscles in the dimorphic claws of adult lobsters are unusual in that, like the claws, they are asymmetrical (Jahromi and Atwood, 1971; Goudey and Lang, 1974; Lang, Costello and Govind, 1977). The crusher claw closer muscle is composed solely of long sarcomere slow fibers. In the cutter claw, the closer muscle contains both fast and slow muscle fibers. This results in a bimodal distribution of muscle fiber types in the cutter (based on sarcomere length), with over 60% being short sarcomere, fast fibers and the remainder being long sarcomere, slow fibers. In the cutter closer muscle, fast and slow fibers are regionally distributed on the inner aspect with fast fibers in the dorsal and central medial sections and slow fibers in the ventral section (Lang *et al.*, 1977).

An interesting feature of the claws is that while they are asymmetric in the adult, they are symmetric during the larval and early postlarval period. Thus in the larval forms, which comprise the first three stages, the bilateral chelipeds are identical, both being cutter-like in appearance. The transformation of one claw of the pair to a crusher cheliped occurs later in development, during the seventh or eighth stage (Herrick, 1896). Presumably, the differentiation of the closer muscle into cutter and crusher types is associated with the change in external morphology of the cheliped which occurs during this time.

The present paper reports on the composition of the bilateral closer muscles in the three larval stages. In each larval stage the bilateral muscles are similar in fiber composition, each having in the first stage mainly short and intermediate sarcomere fibers and relatively few long sarcomere fibers. In the second and third larval stages the closer muscles have an equal distribution of all three fiber types.

Materials and Methods

Newly hatched lobsters were obtained from the Massachusetts State Hatchery on Martha's Vineyard and reared in circulating seawater tanks at ambient temperature (Hughes, Shleser and Tchobanoglous, 1974). Animals were fed brine shrimp several times a day. The first three stages are larval, and during this time the animals are pelagic. Each of the larval stages is easily identifiable by size and external morphology (Herrick, 1896, 1911).

The closer muscle in the claw was prepared as follows: the limb was pinned in a wax bottom dish with the dactyl in the fully open position so that the closer muscle fibers were held in the stretched position. To permit a constant

flow of fixative past the closer muscle, the limb was cut in the carpodite and in the propodite distal to the muscle. The limb was immersed in freshly prepared aqueous Bouin's and a fine jet of the fixative was directed at the cut ends during the first 10 min. After 24 hr in the fixative, the closer muscle was isolated from the limb and stored in 70% alcohol.

The inner aspect of each closer muscle was divided into sections to determine whether there was any regional distribution of fiber types, as there is in the adult (Lang *et al.*, 1977). In the second and third stages, the claw was divided into quadrants and muscle fibers were sampled from each of the four sections. The first stage claw was too small to be similarly divided so fibers were sampled from two areas, dorsal and ventral, for this stage. The method for obtaining the average sarcomere length of a muscle fiber is described in an earlier paper (Lang *et al.*, 1977).

Muscle fiber populations were compared for significant differences (at the 0.05 and 0.01 levels) using the Kolmogorov-Smirnoff two-sample test (Siegel, 1956). Comparisions were made between the two claws for each animal and between the total claw I and claw II populations for each of the four stages examined (stages 1, 2, 3, and late 3). Differences between stages were tested using the total claw I and total claw II populations for each stage.

Results

The paired claw closer muscles in each of the three larval stages of the lobster, *Homarus americanus*, were examined to determine the muscle fiber types that compose them. Using the average sarcomere length of a fiber as a criterion, three fiber types were recognized: short, intermediate and long sarcomere fibers with sarcomere lengths of < 4, 4–6, and > 6 μm, respectively. Table I gives the relative distribution of fiber types in the bilateral closer muscles, and lists the

Table I

Distribution of muscle fiber types in the paired claw closer muscles of larval lobsters.

Stage	Length (cm)	Muscle fiber types based on sarcomere length (μm)					
		Claw I			Claw II		
		Short <4	Intermediate 4–6	Long >6	Short <4	Intermediate 4–6	Long >6
1	0.7	47%	53%	0%	27%	73%	0%
1	0.75	37	57	6	30	60	10
1	0.75	33	63	4	30	70	0
2	0.8	47.5	50	2.5	42.5	52.5	5
2	0.85	42.5	57.5	0	40	60	0
2	0.85	40	57.5	2.5	37.5	55	7.5
3	1	20	60	20	5	75	20
3	1	32.5	55	12.5	30	52.5	17.5
3	1	35	52.5	12.5	17.5	62.5	20
3 (late)	1.05	40	20	40	35	47.5	17.5
3 (late)	1.2	40	37.5	22.5	35	32.5	32.5

FIGURE 1. Frequency histogram of muscle fibers with characteristic sarcomere lengths from the inner aspect of the paired closer muscles of a larval first stage lobster.

closer muscle with the higher percentage of short sarcomere fibers as belonging to claw I. Each larval stage will be described separately.

It should be noted that the muscle fibers in larval claws were classified, on the basis of sarcomere length, into categories similar to those used for the adult claw. However, we presently have no information regarding their physiological and biochemical characteristics. Thus for the larval stages we do not yet know whether the long sarcomere fibers contract more slowly than the short sarcomere fibers. For this reason fibers will be classified simply on the basis of sarcomere length.

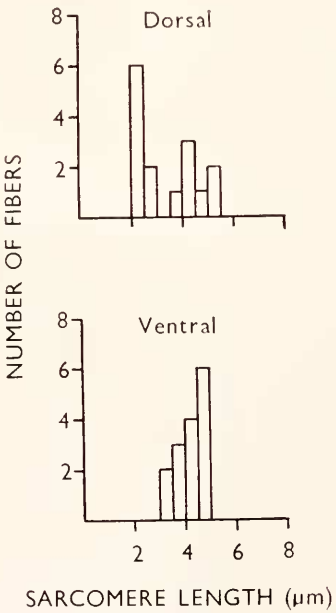

FIGURE 2. Frequency histogram of muscle fiber types (based on sarcomere lengths) showing the regional distribution on the inner aspect of a Claw I closer muscle in a larval first stage lobster.

Stage 1

In first-stage lobsters the small size of the closer muscle (propus length *ca.* 0.9 mm) limited division of the muscle into two sections, dorsal and ventral. From each section 15 fibers were sampled for a total of 30 fibers for each muscle. The relative distribution of fiber types for the three pairs of stage 1 muscles is given in Table I. Each closer muscle of a pair is composed largely of intermediate and short sarcomere fibers and has either few or no long sarcomere fibers. This bimodal pattern is also reflected in a frequency histogram of sarcomere lengths in one muscle pair (Fig. 1) in which peaks typically occur at 2 μm and 4–5 μm. However, when the frequency of fiber types is plotted for the separate muscle areas from which they were sampled (Fig. 2), either the majority (Claw I) or all (Claw II) of the short sarcomere fibers are in the dorsal section. This is similar to the pattern found in the adult cutter closer muscle where the ventral section contains only long sarcomere slow fibers and the dorsal sections primarily short sarcomere fast fibers (Lang *et al.*, 1977).

Statistical comparisons of the claw muscle fiber populations did not reveal any differences between Claw I and Claw II populations. This was true for comparison between claws of each animal as well as between summed Claw I and Claw II populations for three animals (Kolmogorov-Smirnoff two sample test, at 0.05 level).

Stage 2

In stage 2 larval lobsters the propus increases about 30% in length to 1.2 mm; thus, the inner side of the closer muscle could be divided into quadrants. Then fibers were sampled from each of the four sections. The closer muscle at this stage resembles its stage 1 counterpart in that each muscle is composed almost entirely of intermediate and short sarcomere fibers; long sarcomere fibers are entirely absent or, if present, are few in number (Table I). Likewise, each muscle shows a bimodal distribution of fiber types with peaks at 2 μm and

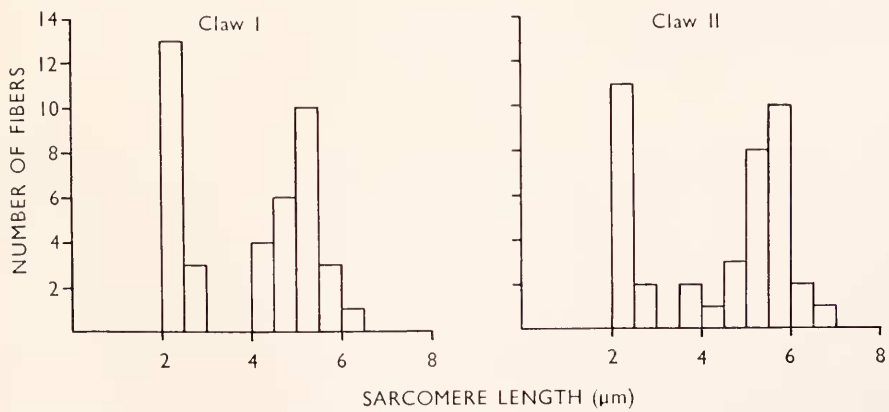

FIGURE 3. Frequency histogram of muscle fibers with characteristic sarcomere lengths from the inner aspect of the paired closer muscles of a larval second stage lobster.

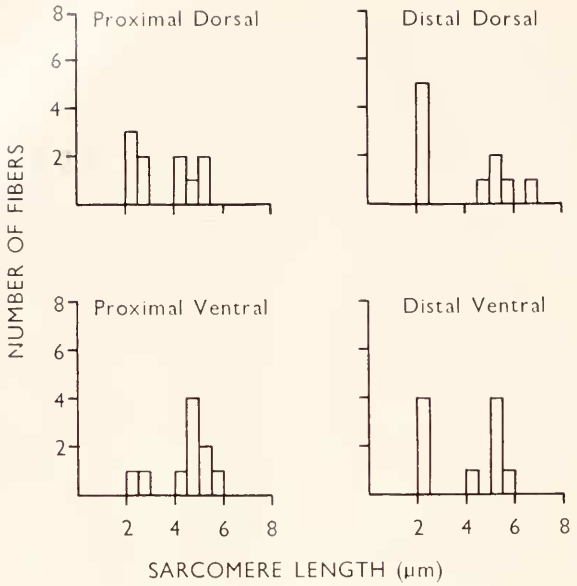

FIGURE 4. Frequency histogram of muscle fiber types (based on sarcomere lengths) showing the regional distribution on the inner aspect of a Claw I closer muscle in a larval second stage lobster.

4.5 μm (Fig. 3). The fiber types do not appear to be regionally distributed, as both types occur in all four sections sampled (Fig. 4). Yet there may be a tendency for the proximal ventral section to contain only intermediate fibers (Fig. 4) as half the closer muscle samples were thus characterized.

Statistical comparisons of claw muscle fiber populations did not reveal any differences between Claw I and Claw II, either for a single animal or for the summed populations of the three animals. Likewise there was no significant difference (at the 0.05 level) between the Claw I and Claw II populations of stage 2 as compared to the respective populations of stage 1.

Stage 3

The sampling procedure for stage 3 closer muscles was similar to that followed for stage 2 muscles. The propus length measures approximately 1.9 mm in stage 3 animals. A total of five animals was examined in this larval stage: three animals were sacrificed when they were midway through this stage and the remaining two when they were near the end of this stage (late 3rd stage in Table I).

The 3rd stage closer muscle differs from its counterpart in the earlier stages in two respects. First, there is an increase in the number of long sarcomere fibers which now comprise between 12.5% and 40% in stage 3 as compared to < 10% in stages 1 and 2 (Table I). This increase is primarily at the expense of the short sarcomere fiber population. Secondly, although there is still a bimodal distribution of fiber types in stage 3 muscles, the second peak (intermediate fibers)

now occurs at 5.5 μm (Fig. 5) as compared to 4.5 μm in the two earlier stages (Figs. 1, 3). The first peak (short sarcomere fibers) occurs at 2 μm in stage 3 muscle (Fig. 5) as in the earlier stages.

A plot of the fiber types in the four sections reveals that all three fiber types occur consistently in the dorsal sections (Fig. 6). In the ventral sections, however, only the intermediate and long sarcomere fiber types occur regularly. There were one or fewer short sarcomere fibers (< 4 μm) present in the ventral sections in three out of the six claws examined. In addition, they comprised 9% of the ventral fiber population but 38% of the dorsal fiber population.

There were no significant differences between Claw I and Claw II populations for individual animals or for the summed population of the three samples. However, the summed population of both Claw I and Claw II were significantly different (at the 0.01 level) from the respective population of stage 2.

Stage 3 (late)

In the late third stage closer muscle, the relative proportions of the three fiber types change so that there is an increase in both short and long sarcomere fibers and a corresponding decrease in intermediate fibers (Table I). The change in the short sarcomere fibers is reflected by their increased occurrence in the ventral areas, while the long sarcomere fibers were observed in higher numbers in all areas. Whether this change in distribution, as compared to earlier third stage animals, is due to the premolt condition or whether it is due to a normal change in fiber types is uncertain. However, the summed Claw I and Claw II populations are significantly different (at the 0.01 level) from their respective stage 3 populations.

Discussion

Neither the claws nor the claw closer muscles in the larval stages of the lobster are differentiated into cutter and crusher types. Both claw closer muscles have short, intermediate and long sarcomere muscle fibers. In the adult, the cutter

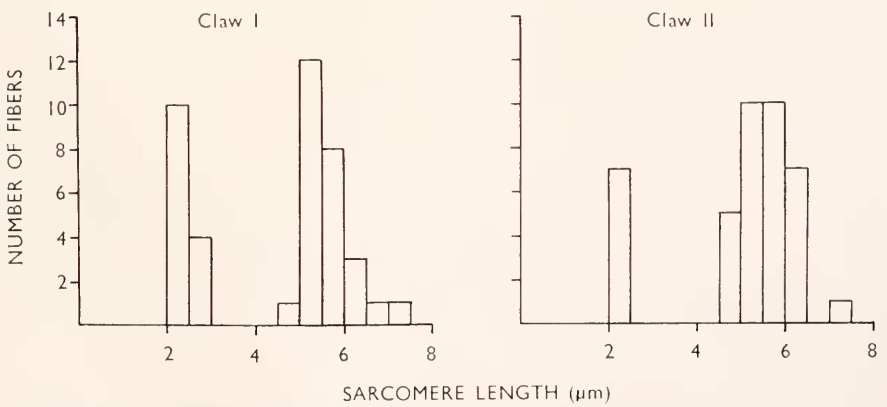

Figure 5. Frequency histogram of muscle fibers with characteristic sarcomere lengths from the inner aspect of the paired closer muscles of a larval third stage lobster.

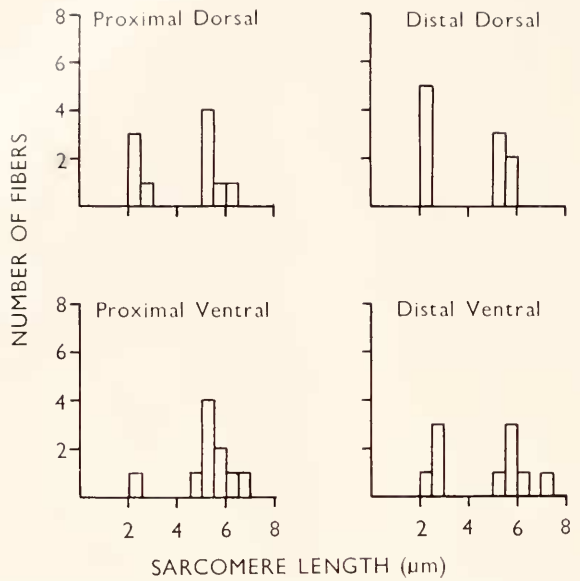

FIGURE 6. Frequency histogram of muscle fiber types (based on sarcomere lengths) showing the regional distribution on the inner aspect of a Claw I closer muscle in a larval third stage lobster.

claw has 60–70% short sarcomere fast fibers and 30–40% long sarcomere slow fibers, while the crusher has all long sarcomere slow fibers; a small proportion (2–4%) of intermediate sarcomere fibers are occasionally found in both muscles (Lang *et al.*, 1977). In contrast the larval muscles possess over 50% ($\bar{X} = 60\%$) intermediate fibers, except in the late third stage where there is a decrease to an average of 34% (Table I). There are few long sarcomere fibers ($\bar{X} = 3\%$) in the first two larval stages, but these increase up to an average of 28% in the late third stage. Short sarcomere fibers, on the other hand, average 38% of the population in the first two larval stages. During the middle of the third stage the short sarcomere fiber population is only 23% of the total, but during the late third stage it again reaches 38% of the total.

The major changes which occur during the larval stages are the transient decrease in the number of short sarcomere fibers in the mid-third stage and the accompanying increase in long sarcomere fibers. During late third stage the short sarcomere fiber population continues to become larger, both these changes being at the expense of the declining intermediate fiber population. From this larval condition, the transformation into the adult state involves the further acquisition of short and long sarcomere fibers only and the concomitant disappearance of the intermediate fibers.

How are these "new" short and long sarcomere fibers acquired? It is possible that they arise *de novo* as fully differentiated short and long sarcomere fibers, or alternatively that they differentiate from existing fibers, including especially the intermediate fibers. On the other hand, it is possible that they arise simply by

differential proliferation of the existing populations of short and long sarcomere fibers, perhaps by fiber splitting. In regard to the first possibility, the abdominal extensor muscles which have morphologically distinct bundles of short sarcomere fibers and long sarcomere fibers exhibit the dichotomy in the earliest stage examined, *i.e.,* in larval lobsters and newly hatched juvenile crayfish (Govind, Atwood and Lang, 1974). However, the sarcomere lengths in these early stages are considerably smaller than those in the adult. Thus, though sarcomere length does increase during development, the differentiation into short sarcomere fibers and long sarcomere fibers is established very early. This apparently does not occur in the claw closer muscles; in the larval stages these muscles are symmetrical and composed of a mixed population of short, intermediate and long sarcomere fibers while the adult closer muscles are asymmetrical, there being a complete loss of short sarcomere fibers in the crusher claw. Thus, while fiber properties of the abdominal muscles are established and fixed during myogenesis, those of the claw muscle are not.

A corollary to this mechanism of genetically specified fiber types would necessitate the loss of the intermediate fibers typically found in the larval forms (Table I) rather than their transformation into short or long sarcomere type. Yet in all the larval lobsters examined in this study, no signs of degenerating muscle fibers were found. Also, the gradual increase in number of long fibers until the late third stage, associated with the shift in sarcomere length peak from 4.5 μm to 5.5 μm in the third larval stage, suggests that the intermediate fibers may transform to long sarcomere fibers by lengthening of their sarcomeres. In fact, the sarcomere length of the long fibers of the abdominal extensor muscles in larval lobsters measures 4–5 μm and subsequently lengthens to the adult size (Govind *et al.,* 1974). In this regard, it is of interest to note that while there is little change in the short sarcomere fiber population from the third to the fourth stage, there is a change in the other fiber populations. Fourth (first juvenile) stage claws average fewer than 5% intermediate fibers. Thus, there appears to be a further shift of the intermediate fibers to long sarcomere fibers at or shortly following the fourth molt (to the fourth stage) (in preparation). This problem of transformation of muscle fiber types does not appear to have been previously studied in invertebrate muscle, but it has been shown to occur in mammalian and avian muscle (for reviews see Harris, 1974; Gutmann, 1976).

The data support the hypothesis that there is a transformation of intermediate sarcomere fibers to long sarcomere fibers. Although care was taken to fix all claws in the same fully open position, it is possible that some differences were due to anatomical changes in the claw. This seems improbable for the changes observed between mid-third and late-third stage. Claw shape seems unlikely to change during the intermolt period, yet there was a statistically significant difference between claws from these two ages. However, definitive proof must await ultrastructural studies which will permit analysis of other characteristics of the muscle fibers including sarcomere length, A-band length, sarcoplasmic reticulum, etc.

While there may be transformation of intermediate fibers to long sarcomere fibers, it would appear that the same is not true in regard to transformation of intermediate fibers to short sarcomere fibers. If intermediate sarcomeres did

transform by decrease of sarcomere lengh, it would also necessitate addition of sarcomeres to maintain a constant muscle fiber length. Furthermore, in the majority of larval muscles the short sarcomere fiber population is distinctly separated from the intermediate fiber population. The short sarcomere fibers range between 2–3.5 μm with the majority at 2 μm; the intermediate fibers range between 4–6 μm. If the intermediate fibers were redifferentiating into short sarcomere fibers, a continuous range between these fiber types (2–6 μm) without any distinct breaks might be expected.

Thus, the present data support the hypothesis for transformation of intermediate fibers to long sarcomere fibers. These two fiber types form a continuous population with no apparent bimodal distribution. However, it is clearly desirable to have ultrastructural evidence of these fiber populations, to unequivocally determine that they form a continuum.

We thank John Hughes, Director of the Massachusetts State Lobster Hatchery, Martha's Vineyard, for generously supplying us with larval lobsters and advising us in their maintenance. This work was supported by grants from: NIH and Muscular Dystrophy Association of America to F. L. and by NRC and Muscular Dystrophy Association of Canada to C. K. G.

Summary

1. The closer muscles of the paired claws (chelipeds) of lobsters were characterized according to the distribution of short, long and intermediate sarcomere muscle fibers during the three larval stages.

2. Unlike the adult lobster, where the claws and closer muscles are asymmetrical, the claws and closer muscles of the larval stages are symmetrical.

3. In the first and second larval stages, the closer muscle is composed of over 50% intermediate sarcomere fibers, 30–40% short sarcomere fibers and less than 10% long sarcomere fibers.

4. By the late third stage the long sarcomere fibers have increased to a maximum of 40% with a corresponding decrease in number of intermediate fibers.

5. Thus, at the end of the last larval stage, the closer muscles are symmetrical, with muscle fibers about equally distributed among short, intermediate and long sarcomere fiber types.

6. The data are consistent with the hypothesis that intermediate fibers are transformed into long sarcomere fibers but not into short sarcomere fibers.

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